Refer to: Rosenberg RN, Campbell J: Myasthenia gravis—University of California, San Diego (Specialty Conferences). Calif Med 118:18-23, Jan 1973

# Specialty Conference

# Myasthenia Gravis

*Participants:* ROGER N. ROSENBERG, M.D.,
JAMES CAMPBELL, LT COMM, USN, MC

*Taken from the weekly Pediatric Grand Rounds held at the University Hospital of San Diego County, University of California, San Diego, School of Medicine, June 20, 1972*

DR. CAMPBELL:* The patient, a Filipina, was first seen at the U.S. Naval Hospital in San Diego in March of 1971, when she was 7 years, 3 months of age. At that time, she complained of diplopia. She had no other symptoms of weakness. There was no difficulty in swallowing and no weakness referable to muscles of the extremities.

*Physical Examination*

She was well developed, slender and intelligent. Neurological examination revealed bilateral ptosis and bilateral symmetrical ophthalmoparesis in all directions of gaze. She had diplopia on far lateral gaze bilaterally. Examination of the other muscle groups for tone, bulk, strength, and reflexes was entirely normal. She was given edrophonium chloride (Tensilon®) 0.1 cc (1 mg) intravenously and complete disappearance of ptosis and ophthalmoparesis followed. A diagnosis of juvenile myasthenia gravis was made.

The treatment of the patient was titrated to a dose of of 20 mg of pyridostigmine (Mestinon®) three times a day. Both the ptosis and the ophthalmoparesis responded well.

In May, 1971, the patient was again admitted because of a nasal quality to her speech, drooling, and dysphagia. The amount of pyridostigmine was increased to 60 mg every 8 hours. She did well for four weeks, but in June generalized weakness, abdominal pain and increased salivation developed. She was readmitted and placed on a respirator for 72 hours. On discharge she was given pyridostigmine 300 mg four times a day. In September of 1971 she was again admitted for respiratory insufficiency thought to be related to cholinergic crisis, and again was placed on a respirator. In September thymectomy was performed. After the operation she remained on a respirator for five days, and administration of pyridostigmine 30 mg every 5 hours and ephedrine sulfate 12.5 mg three times a day was begun again. Gross and microscopic examination of the thymus was entirely normal. In February, 1972, an influenza-like illness developed. At that time the patient was taking pyridostigmine 60 mg every 6 hours. On March 2, the body temperature rose to 40.6° C (105° F) respirations were shallow and the patient cyanotic. She was admitted to the U.S. Naval Hospital where tubes were placed and she was put on a respirator. Cardiac arrest occurred and all resuscitative measures were unsuccessful.

Autopsy revealed moderate lymphorrhage formation throughout the entire myocardium, and extensive infiltration was noted in skeletal muscle.

Reprint requests to: R. N. Rosenberg, M.D., Chief, Division of Child Neurology, University of California, San Diego, School of Medicine, P. O. Box 109, La Jolla, Ca. 92037.

*James Campbell, Lt. Comm., U.S.N. Medical Corps, Pediatrics, U.S. Naval Hospital, Balboa, San Diego, California.

## Discussion

DR. ROSENBERG:* Myasthenia gravis is a neurological disorder of unknown cause in which there is weakness, especially of muscles innervated by cranial nerves, due to a neurophysiological impairment of neuromuscular transmission. The weakness is corrected by cholinergic drugs. The disease has been reported as occurring from the neonatal period through the ninth decade of life. Myasthenia gravis unassociated with a thymoma occurs most frequently in the third, fourth, and fifth decades of life in women and in the sixth and seventh decades in men. In contrast, myasthenia gravis associated with thymoma is most frequent in the sixth and seventh decades of life in both sexes. It is a disorder which occurs in children, and neonatal myasthenia occurs in about 10 percent of infants born of myasthenic mothers. Cases such as the one under discussion, that of a 7-year-old girl with seriously progressive myasthenia gravis, are seen with some regularity on neurological services.

### *The Myoneural Junction*

The neromuscular junction is the anatomic site and a neurophysiological impairment in neuromuscular transmission is the basis for weakness in this disease. Thus, it becomes important to review the anatomic structures and the neurophysiology and biochemistry of events at the neuromuscular junction to relate them to myasthenia. The motor nerve axon develops into an expanded nerve terminal at the point of innervation with a voluntary striated skeletal muscle fiber, and in the presynaptic nerve terminal one can readily identify two important structures in electronmicrographs. The first are the 600 Å cholinergic vesicles containing between 10 and 50 thousand molecules of acetylcholine. The other important structure is the mitochondrion, which not only provides adenosine triphosphate (ATP) for energy for the biochemical processes of neuromuscular transmission but specifically is the site of the pyruvic acid dehydrogenase complex and acetyl-coenzyme A synthetase, two enzymes required for the generation of activated acetate or acetylcoenzyme A; this important precursor substrate is utilized with choline to generate acetylcholine presynaptically in the cytoplasm of the nerve terminal. The cytoplasmically synthesized acetylcholine is then taken up into the cholinergic vesicles for storage. When the motor axon is electrically depolarized, vesicle-stored acetylcholine is released by exocytosis through a series of biochemical steps which remain unknown. Free acetylcholine then transverses the 200 Å gap between the pre-membrane and post-membrane to arrive at the postjunctional membrane receptor site, depolarizing the membrane of the muscle fiber and generating a discrete muscle miniature end plate potential. Somewhere in this sequence of events in myasthenic patients there is a defect or defects which underlie the impairment in neuromuscular transmission.[1,2]

The mechanisms by which acetylcholine is stored in the synaptic vesicle are complex. The molecular defect in myasthenia may reside even within the vesicle itself. Information on the cholinergic synapse in the superior cervical ganglion of cats suggests that there is a readily releasable acetylcholine fraction, and that the acetylcholine that is snythesized last is released first. A depot pool of acetylcholine is then activated subsequent to the total release of the readily releasable fraction. There is also a stationary pool of acetylcholine which is even less accessible for release. Thus, it is possible that the molecular defect in myasthenia is in the activation of acetylcholine from one pool to the other within the nerve terminal. This would lead to an inability to release acetylcholine and make it unavailable to the postjunctional receptor site.

The biochemical events of acetylcholine synthesis and its storage in a vesicle might be entirely normal in myasthenia, and the impairment in neuromuscular transmission might result from impairment in the ability for acetylcholine to be retrieved from the cholinergic vesicle. It has been suggested that there is a specific mechanism by which the vesicle moves down from the depths of the nerve terminal toward the prejunctional membrane and fuses with the plasma membrane of the nerve terminal. With fusion, the acetylcholine from the vesicle is deposited into the cleft between the pre-junction and the post-junction by a process referred to as exocytosis. It is possible that this series of steps might be impaired, either mobilization down toward the pre-junctional membrane of the vesicle, or impairment of fusion of the vesicle with the prejunctional membrane and subsequent release of

*Roger N. Rosenberg, M.D., Associate Professor of Neurosciences and Pediatrics.

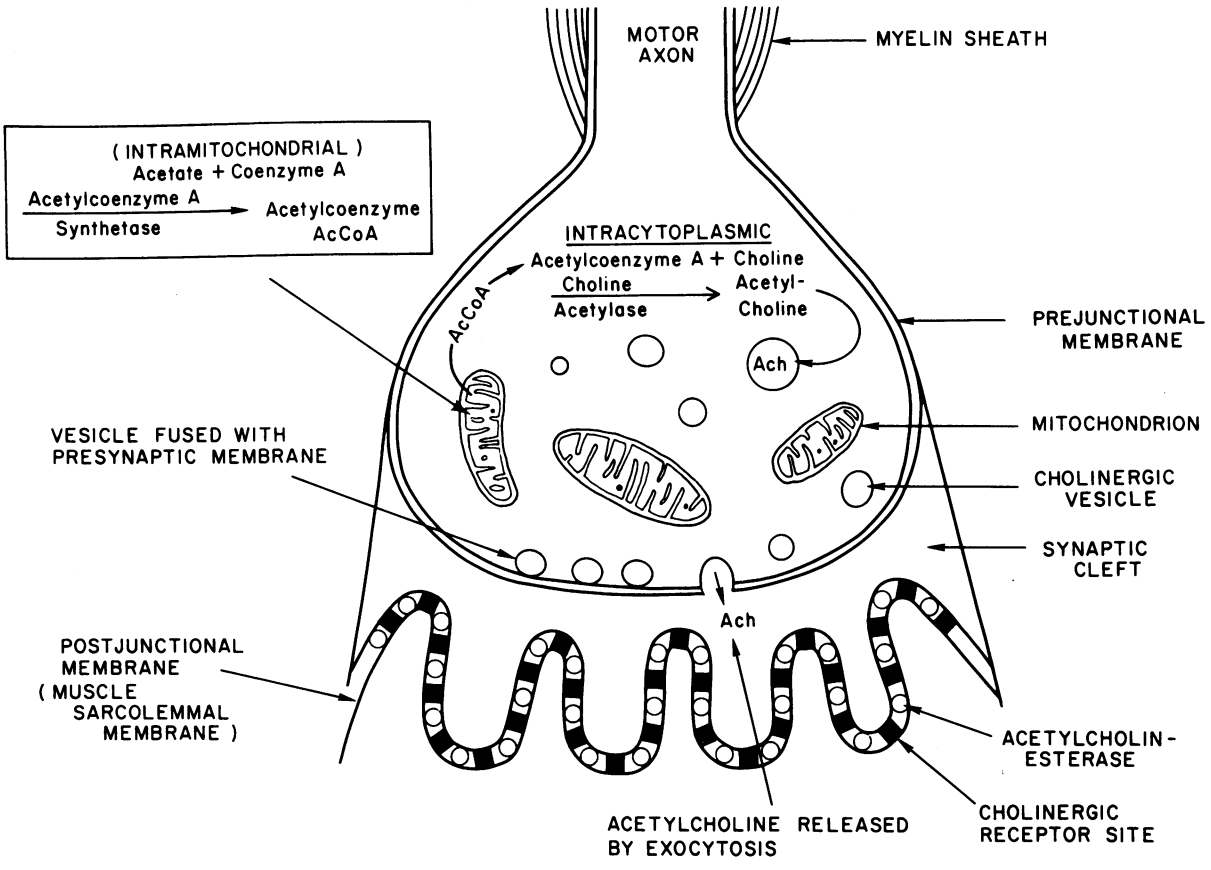


**Figure 1.—The myoneural junction is illustrated indicating the important structures and sequence of events involved in the metabolism of acetylcholine.**

acetylcholine. The impairment in neuromuscular transmission theoretically could result because of reduced availability of acetylcholine from impairment of any one of the steps mentioned.

The only biochemical steps in acetylcholine metabolism which are described in the normal neuromuscular junction are for its synthesis. This involves the generation of acetyl-coenzyme A from acetate, which is catalyzed by acetyl-coenzyme A synthetase, an intramitochondrial enzyme. This is followed by the choline acetylase reaction in which acetylcholine is synthesized from acetyl-coenzyme A and choline, and this occurs in the cytoplasm of the nerve terminal. Acetylcholine is then taken into the cholinergic vesicle and stored (Chart 1).

### *Effects of Acetylcholine on the Post-junctional Membrane*

Acetylcholine results in the production of miniature end plate potentials (MEPPS) at the neuromuscular junction. The miniature end plate potential at the neuromuscular junction is analogous to the excitatory post-synaptic potential which occurs within the central nervous system. Miniature end plate potentials are produced by the quantal release of acetylcholine. The dogma is that one vesicle contains one quantum of acetylcholine, and, when released and combined with the post-junctional membrane receptor site, produces a permeability change equivalent to one miniature end plate potential. Miniature end plate potentials then summate in time and space to produce the all-or-none propagated muscle action potential which then sweeps longitudinally across the sarcolemma muscle membrane and into the depths of the muscle via the transverse T-tube system. The normal miniature end plate potential is one millivolt. The myasthenic miniature end plate potential is approximately 0.2 millivolt. The frequency of occurrence of miniature end plate potentials in myasthenia is entirely normal.[2]

A reduced amplitude of a miniature end plate potential might result due to impaired rate of synthesis of acetylcholine relative to its utilization, impaired intake of acetylcholine into the cholinergic vesicle, impaired release of acetylcholine from the vesicle, inactivation of acetylcholine within the cleft between the pre-membrane and the post-membrane, or a decrease in sensitivity of the receptor site itself to acetylcholine. Further, small miniature end plate potentials might be produced because of abnormal synthesis of a false transmitter which competes with acetylcholine for the receptor site. It is also possible that the small miniature end plate potential results from increased activity of acetylcholinesterase, making less acetylcholine available to the adjacent receptor site. The mechanism of pathogenesis is unknown, but it is clear that we are dealing with a selective abnormality in one cholinergic synapse, the voluntary striated neuromuscular junction.

*Clinical Manifestations*

There have been several landmarks in our understanding of myasthenia since Sir Thomas Willis first described this disease in 1672.[3] In 1934, Mary Walker reported that myasthenia gravis resembled very much the clinical syndrome of curare poisoning, and she suggested that the use of anticholinesterase drug might be helpful in managing patients with myasthenia gravis, as it is in curarized persons. In 1941, Harvey and Masland reported the defect in neuromuscular transmission. Demonstration of the impaired neuromuscular transmission is a valuable clinical tool which can be used in the day to day evaluation of myasthenia. A stimulating electrode is placed on the nerve and a recording electrode on the muscle. The nerve is stimulated at 3 to 20 cycles per second, and the amplitude of the evoked muscle action potential is recorded with repetitive stimulation. In myasthenia there is a very profound, very quick fall-off in the amplitude of the evoked muscle potential with repetitive stimulation. This is the hallmark of the clinical diagnosis of myasthenia.[1] The improvement in amplitude of the muscle action potential with rest or the administration of an anticholinesterase drug is also a characteristic of this disease. Neurophysiological parameters correlate well with improved strength in a patient who receives an anticholinesterase drug. These events may occur quickly and dramatically following the administration of edrophonium choride (Tensilon®).

The involvement of musculature innervated by cranial nerves is common in myasthenia. Weakness of extraocular muscles, ptosis, facial weakness, a sneer instead of a smile, weakness on chewing and swallowing, and weakness of the extensors and flexors of the neck are common presenting manifestations in myasthenia gravis. Weakness of muscles innervated by cranial nerves is commonly seen in association with generalized weakness. To confirm that the occurence of this weakness is due to myasthenia, one should do the following: 1) perform a Tensilon test, using 10 mg to 15 mg of edrophonium chloride injected over a 90-second period and observe for a quick but transient improvement in strength; 2) determine if there is impairment in neuromuscular transmission by repetitive stimulation of a peripheral nerve and recording of the amplitude and frequency of the evoked muscle action potentials. The muscle tested, of course, should be a muscle which is clinically affected.

*Treatment*

Once the diagnosis of myasthenia gravis has been established, the patient should be treated with longer acting anticholinesterase drugs such as prostigmine or pyridostigmine. After the appropriate drug dosage has been achieved, the patient should again be rechallenged with a Tensilon test to see if that dose of anticholinesterase drug produces maximal effect. It should be emphasized that not enough acetylcholine at the receptor site results in weakness, as does too much acetylcholine.

The features differentiating myasthenic deterioration or myasthenic crisis from cholinergic deterioration or cholinergic crisis may be difficult to distinguish. The occurrence of salivation, small pupils, diaphoresis, goose bumps, abdominal cramps, and bladder cramps, associated with progressive weakness strongly indicates excessive acetylcholine. A Tensilon test performed in such conditions may be dangerous. The patient's muscle function can rapidly deteriorate to the point of respiratory insufficiency. If the patient is progressively becoming weaker without associated cholinergic autonomic manifestations and dra-

matically improves with Tensilon, it indicates the presence of myasthenic crisis or insufficient acetylcholine at the neuromuscular junction cholinergic-receptor site.

*Pathogenesis of Myasthenia*

What is the current information to indicate what the biochemical defect may be in this disease? It is possible that the impairment in neuromuscular transmission results from a hemicholinium-like defect. Hemicholinium is a choline analog which impairs the rate of synthesis of acetylcholine presynaptically by restricting the availability of choline through blocking its re-uptake. Hence a motor axon which is rapidly depolarizing and releasing acetylcholine may rapidly lose its capacity to synthesize acetylcholine at a rate commensurate with its needs. The second possibility is a botulinum toxin-like defect. Botulinum toxin is a very potent agent which prevents the presynaptic release of acetylcholine. Either drug would have the effect of reducing availability of acetylcholine to the cholinergic receptor protein. Acetylcholine may not be the only transmitter synthesized; a false transmitter might be synthesized and incorporated into the vesicle, displacing acetylcholine. When such a vesicle reached the prejunctional membrane, fused with it, and released its contents by exocytosis, it would release the competitive false transmitter as well. Thus, a false transmitter might impair neuromuscular transmission by inhibiting acetylcholine entry into the vesicle or competing with acetylcholine for the cholinergic receptor site post-synaptically. Another possibility is the production of an inhibitor which binds to released acetylcholine in the cleft, producing inactivated acetylcholine. Acetylcholinesterase might be more active than normal in the myasthenic patient, producing increased hydrolysis and less available acetylcholine for the receptor site.

The sensitivity of the cholinergic receptor site for cholinergic drugs has been directly measured and is normal. This has been measured by the iontophoresis of acetylcholine as well as the administration of decamethonium or carbachol onto *in vitro* neuromuscular junctions. Thus, from this information the impairment of neuromuscular transmission appears not to be a post-synaptic event but rather a pre-synaptic one.[2]

What is known about the anatomy of the presynaptic nerve terminal in normal and in myasthenic persons, and what is known about the presynaptic events of acetylcholine metabolism in this disease? The vesicle diameter in myasthenic patients is normal, approximately 568 Å. Further, the density of vesicles is normal in this disease. There are about 50 vesicles per square micron in myasthenic persons. The nerve terminal presynaptically is significantly reduced in surface area, however. The average nerve terminal in normal persons is about 3.92 ± 0.4 $\mu^2$ and in myasthenic persons is about 2.28 ± 0.2 $\mu^2$, or about 40 percent smaller.[1]

The normal size of the vesicle is one indirect piece of evidence to indicate that the defect is not a disorder in the rate of acetylcholine synthesis. If a motor neuron is stimulated at a high frequency in the presence of hemicholinium to reduce the availability of choline for acetylcholine synthesis, the amplitude of the muscle action potential will fall off rather rapidly, a condition analogous to myasthenia. However, the size of the vesicles is also reduced by about 40 percent in volume and diameter in this experimental situation.[5] The vesicle diameter in myasthenia is normal, however, which is one important bit of information against impaired synthesis. Also, if there were impaired synthesis, one might expect a progressive fall in the amplitude of the miniature end plate potential with repetitive high frequency stimulation. There is in myasthenia, however, a constant reduction in miniature end plate potential amplitude and it does not decrease with further stimulation.[2]

I recently had opportunity to assay the rate of synthesis and rate of hydrolysis of acetylcholine directly by radioisotopic chromatographic assays, utilizing homogenates made from deltoid muscle from normal and myasthenic individuals. The mean specific activity of choline acetylase from 11 controls was 7.6 ± 0.7 nanomoles of acetylcholine formed in 10 minute per mg of protein at 37° C, pH 6.8; and in 10 myasthenic persons it was 5.4 ± 0.7 units, or 30 percent less than in normal controls.[4,5]

I interpret these data as indicating that the defect is not in synthesis of acetylcholine in this disease. I say this for two reasons: (1) there was overlap to a considerable degree in choline acetylase values between normal and myasthenic persons, and (2) there was no correlation in

specific activity in deltoid muscle homogenates from weak and non-weak myasthenic muscle. I think these data reflect the atrophied nerve terminal rather than an impaired rate of synthesis of acetylcholine. The nerve terminal is about 40 percent smaller in myasthenia, and the mean specific activity for choline acetylase was about 30 percent less. Product identification was also carried out by use of labeled choline or labeled acetate, and in both instances the only product produced in the normal and myasthenic homogenate was acetylcholine. This rules out the possibility of a false transmitter which might be a choline analog such as succinylcholine or an acetate analog such as acetylcarnitine. It still leaves open the possibility that there is a noncholine or non-acetate analog which is being synthesized in this disease.

It may be that myasthenia is an autoimmune disease and that the neurophysiological and biochemical changes that I have described may be phenomena secondary to a basic immunological defect.[6,7] Thymic hyperplasia develops in about 80 percent of myasthenic patients, and in about 10 percent a true thymoma develops. Myasthenia develops in about 30 percent of patients who have a thymoma. Furthermore, myasthenia has been associated with other more classically accepted autoimmune diseases such as rheumatoid arthritis, lupus erythematosus, thyroiditis, and polymyositis. Anti-muscle antibodies have been described in about 20 percent of myasthenic patients. Thymectomy may significantly improve a patient or produce a complete remission. A recent study from the Mt. Sinai Hospital in New York indicated that total remission or a significant improvement occurred in 75 percent of myasthenic patients after thymectomy, even if they had the disease more than five years. Many patients had to be followed for some time after thymectomy, sometimes as long as ten to twelve years, before total remission occurred.[8,9] More recently, the use of ACTH and prednisone have been reported to help selected patients significantly. Thymectomy, ACTH and steroids may all be affecting the same immunologic defect at different points.[1] It is possible that there is a clone of B lymphocytes (non-thymus derived) which is producing a toxin or antibody against the neuromuscular junction, resulting in impaired myoneural transmission. One possibility is that this lymphocytic factor is impairing the uptake or release of acetylcholine presynaptically in view of the finding of normal postsynaptic cholinergic sensitivity and normal presynaptic rate of synthesis. The T or thymus derived lymphocyte is known to have a helper effect on increasing the effectiveness of competent immunoglobulin synthesis by the non-thymus derived B lymphocyte. The long time it takes for a response from thymectomy may be the time it takes to dilute out the thymus derived lymphocyte and its known helper effect on stimulating immunoglobulin synthesis by the B lymphocytes.

The management of a myasthenic patient whose condition is deteriorating should include early thymectomy followed by careful titration of anticholinesterase drugs to the maximum therapeutic response. Selected patients may require multiple courses of ACTH to maintain clinical remission, and some patients appear to respond to long-term maintenance with alternate day prednisone. Thymectomy, ACTH, and steroids are effective, as they may directly interfere with the autoimmune process which underlies this disease.

## REFERENCES


1. Fields WS (Ed): Myasthenia gravis. Ann NY Acad Sci 183:1-386, 1971

2. Elmqvist D: Neuromuscular transmission with special reference to myastenia gravis. Acta Physiol Scand 64 (Suppl 249):1-34, 1965

3. Willis T: Two discourses concerning the soul of brutes . . . the first is physiological . . . the other is pathological, which unfolds the diseases . . . towit, the plan for nervous stock . . . Englished by S. Pordage, London, printed for Thomas Dring, Ch. Harper and John Leigh, 1683

4. Rosenberg RN, Dalessio DJ, Tremblay J, et al: Kinetics of acetylcholine synthesis and hydrolysis in myasthenia gravis. Science 173: 644-45, 1971

5. Gentner D, Rosenberg R: Choline acetyltransferase and acetylcholinesterase and their role in the cause of myasthenia gravis. Arch Neurol 27:521-525, 1972

6. Wolf WM, Rowland LP, Schotland DL, et al: Myasthenia as an autoimmune disease—Clinical aspects. Ann NY Acad Sci 135:517-535, 1966

7. McFarlin DE: Myasthenia gravis. *In* Samter M (Ed): Immunological Diseases, 2nd Ed. Boston, Little, Brown Co., 1971, pp 1150-1171

8. Papatestas AE, Alpert LI, Osserman KE, et al: Studies in myasthenia gravis—Effects of thymectomy. Am J Med 50:465-74, 1971

9. Alpert LI, Papatestas A, Kark A, et al: A histologic reappraisal of the thymus in myasthenia gravis. Arch Pathol 91:55-61, 1971